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Antibacterial and anti fungal efficacy of some medicinal plants used in Indian herbal medicine

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Abstract
The present study was undertaken to study the antimicrobial and anti fungal activity of selected medicinal plants *viz, Aegle marmelos, Butea monosperma, Comnifora wighti, Holostemma ada-kodien, Decalepis hamiltonii, Gloriosa superba, Gymnema sylvestre* and *Santalum album* were studied from Indalwai forest of Nizamabad. All the bacterial cultures are human pathogenic and were procured from Primer Biotech Research Centre, Hyderabad. The micro organisms selected for the present study are; *E. coli, K. pneumonia, P. aeruginosa* (Gram -ve); *P. vulgaris, S. aureus, B. subtilis*, and *E.s faecalis* (Gram +ve). The present study indicated the anti-microbial and anti fungal activity of methanol leaf extracts of the selected medicinal plants. The present study confirms the extracts of selected medicinal plants can be employed as antimicrobial agents in formulation of the novel drugs. Further it necessitates the pharmacological evaluation.

Keywords: antimicrobial, anti fungal, Gram +ve, Gram –ve, Ampicillin and Itraconazole

Introduction
Ayurveda, Siddha and Homoeopathy are the basis of Indian traditional system of medicine. Ayurveda is the oldest among all these practices and offers a holistic approach and it is popular in practice. Most of the great works of Vedic era has been destroyed by invaders and still a few likes of Charaka Samhita, the Sushruta Samhita and the Bhela Samhita are available. The great sages like Dhanvanthari, Charaka, and Susrutha practiced the traditional medicine and it spread across the globe from India.

Medicinal plants contain some inherent active ingredients that are used to cure disease or pain. They represent a consistent part of the natural biodiversity and the plants are the precursor sources for at least a quarter of therapeutic medicines. Most of the population *i,e* almost more than 80% of the population of third-world countries relied on traditional system of medicine according to WHO (Vines, 2004) [1].

Owing to the minimal toxicity, cheap availability and pharmacological activity of the phytochemicals with already proven therapeutic value are preferred by mankind in relation to the synthetic drugs. Synthetic drugs may be a subject of adulteration and side effects also cannot be ruled out. Secondary metabolites secreted by plants as part of the metabolism are being exploited as sources of medicinal compounds (Lis-Balchin and Deans, 1997) [2]. Plants remain an indispensible in combating illness due to the presence of bioactive compounds. Plants have been explored for drugs for the therapeutic use, additives in food, agrochemicals and industrial chemicals and many others (Charu *et al.*, 2012, Habila *et al.*, 2011) [3, 4].

Since time immemorial, medicinal plants have been exploited for their medicinal use. Nature has been the source a large number curative compounds in addition to the resources. Increased resistance by microbial pathogens to the accessible medications has brought an endangered situation, over utilization of anti-infectious agents, poor sterile conditions etc are also favored the situation.

John De Britto *et al.*, (2011) [14] assessed the methanol and fluid concentrates of leaves of *Acalypha indica*, *Aerva lanata*, *Phyllanthus amarus*, *Phyllanthus emblica*, *Cassia auriculata* and *Caesalpinia pulcherima* were screened for antibacterial investigations and reported that methanol concentrates of *Acalypha indica*, *Aerva lanata* and *Phyllanthus amarus* displayed clear zone of restraint against the test bacterium *Xanthomonos campestris* where MIC (minimum inhibition concentration) estimation of *Aerva lanata* was 32 µg/mL. Ushimaru

et al., (2007) [6] worked on the *in vitro* antimicrobial movement of methanolic concentrates of few plants on *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Enterococcus* species and it was found that the methanolic concentrates of *Caryophyllus aromaticus* displayed the most astounding enemy of *Staphylococcus aureus* movement and furthermore successful against all the bacterial strains tried. An examination was done by Chakraborthy (2008) [7] to assess the antibacterial movements of *Calendula officinalis* Linn. The ether, chloroform, and ethanol plant extracts were screened for its antibacterial and antifungal action utilizing agar well diffusion approach. The living being was *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus niger*. The separates indicated antibacterial movement however none of the concentrates demonstrated antifungal movement. Asha Devi *et al*., (2009) [8] investigated on extracts of *Acorus calamus* and found that rhizomes have bioactive compounds against contagious and yeast. Both rhizome and leaf separates exhibited significant antifungal and hostile to yeast exercises, they didn't demonstrate any antibacterial action with the exception of that of *Escheresia coli*.

Therefore, there is a need for new antibiotics, and medicinal plants may offer another wellspring of antibacterial specialists. Bacterial protection from accessible antimicrobials has been recorded; resistance to antibiotics by bacteria is a growing concern. Ethno pharmacologists, botanists, microbiologists and scientific experts are attempting to find phytochemicals, which could be produced for treatment of irresistible maladies (Kavitha and Padma, 2008) [9]. The present study was planned to know the antimicrobial and fungal activities of selected medicinal plants against some bacterial pathogens.

Materials and Methods

Collection of plant material

Leaves were collected from selected medicinal plants and processed for anti bacterial and anti fungal studies.

Bacterial cultures used

All the bacterial cultures are human pathogenic and were procured from Primer Biotech Research Centre, Hyderabad. The micro organisms selected for the present study are; *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* (Gram negative bacteria); *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis* (Gram positive bacteria).

The organisms obtained from the laboratory stock were sub cultured into a sterile nutrient broth and incubated at 37ºC for 18-24 h a day prior to the experiment. The culture growth obtained was used as inoculums for the antibacterial testing.

Fungal Cultures Used

All the fungal cultures selected for the present study were procured from Primer Biotech Research Centre, Hyderabad. *Candida albicans* and *Aspergillus niger* were selected for the present investigation to know the anti fungal activity. Two days before the testing, the culture is prepared by inoculating the fungus from master culture into Potato Dextrose Agar (PDA) medium and incubated for 48 hours at room temperature.

The standards Ampicillin, Itraconazole were used as standards for antibacterial and antifungal studies respectively. All the test compounds (controls) were tested at 250 µg/mL. All the plant leaf extracts of methanol were tested at 25 µg/mL, 50µg/mL, 75 µg/mL, 100 µg/mL. Paper disk of 6 mm diameter and 2mm thickness was used for the test. These disks were sterilized by autoclaving at 121ºC (15 lbs psi) for 15 minutes.

Preparation of Leaf extracts

The leaves of *Aegle marmelos*, *Butea monosperma*, *Comnifora wighti*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* were dried under shade and made to a fine powder. The powder (100 grams) were extracted with methanol and dried for 3 hours.

Culture media provides all essential nutrients for the growth of microorganism. Luria Broth (LB) Agar was used to inoculate bacterial strains and PDA (Potassium-dextrose agar) medium was used for fungal strains. Nutrient media prepared was sterilized by autoclave at 121ºC for 20 mins at 15 lbs pressure.

Procedure

Petri dishes were filled up to of 3-4 mm with a nutrient agar medium. This poured medium was allowed to set and then inoculated with susceptible test organism culture using cotton swab under aseptic conditions under laminar air flow unit. Each plate was divided into four equal portions along the diameter. Each portion was used to place one disk. Four disks of each sample were placed on four portions using sterilized forceps. Two disks were placed one each with ciprofloxacin disk and a disk impregnated with the solvent. The petri dishes were incubated at 37 °C for 24 h for bacterial culture and incubated for 28 ºC for 4 days for fungal culture. Diameter of the zone of inhibition was measured. The diameter obtained for the test samples were compared with that produced by standards.

Results and Discussion

All the bacterial pathogens selected viz, *E.coli*, *Klebsiella pneumonia Pseudomonas aeruginosa* (Gram -ve), *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis* (Gram +ve) and fungal pathogens *Candida albicans* and *Aspergillus niger* were tested against *Aegle marmelos*, *Butea monosperma*, *Commifora wighti*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* plant extracts of the concentration of 25 µg/mL, 50 µg/ml, 75 µg/ml, 100 µg/mL impregnated on discs.

All the test compounds *i, e* (Ampicillin-antibacterial, Itraconazole-antifungal) were tested at 250 µg/mL concentration.

The present study indicated the anti-microbial activity of methanol leaf extracts of the selected medicinal plants (Table no's 1-8 and figure no's 1-8). The present study indicated the possible antimicrobial activities. Further it requires several detailed pharmacological assessment. The present findings are in concurrence to several previous reports, (*Xanthomonas campestris*; Satish *et al*., 1999) [10]. The present findings illustrated that the Gram-positive bacteria were more susceptible than Gram-negative and this can be attributed to the difference in composition and structure of cell wall (Pankaj *et al*., 2008) [11].

Table 1: Zone of inhibition of antibacterial activity of selected medicinal plants against *E. coli* (Values were with mean ± SE of three separate experiments).

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	1 ±0.1	2.5±0.2	2.9±0.2	3.5±0.1
Butea monosperma	0.5 ±0.3	1.2 ±0.4	1.8±0.3	3.2±0.5
Commifora wighti	1±0.2	1.5±0.5	1.9±0.5	2.2±0.2
Holostemma ada-kodien	1.1±0.5	1.5±0.4	1.7±0.4	2.2±0.4
Decalepis hamiltonii	1±0.4	2.5±0.4	2.5±0.4	3.0±0.7
Gloriosa superba	1.3±0.4	1.8±0.6	2.2±0.8	2.8±0.5
Gymnema sylvestre	0.6±0.1	1.4±0.7	1.8±0.5	2.1±0.4
Santalum album	0.2±0.1	0.6±0.4	0.9±0.3	1.3±0.2

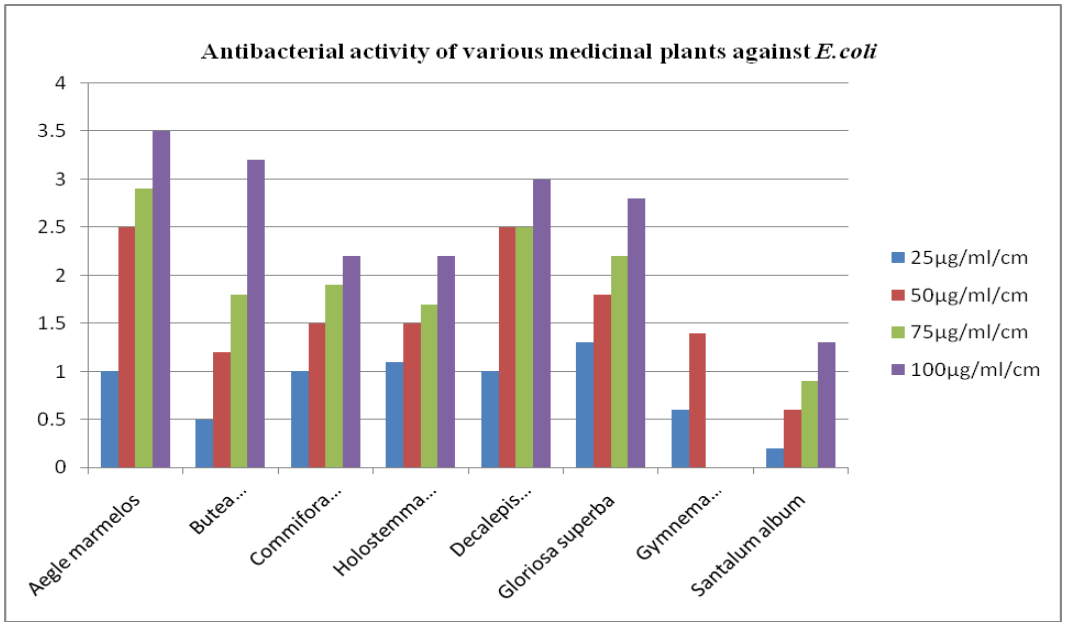

Fig 1: Antibacterial activity of selected medicinal Plants against *E. coli*

Table 2: Zone of inhibition of antibacterial activity of selected medicinal Plants against *K. pneumonia*

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	0.8 ±0.2	1.2±0.4	1.8±0.2	2.1±0.4
Butea monosperma	0.3±0.1	0.6±0.2	1.1±0.1	1.8±0.2
Commifora wighti	0.9±0.4	1.3±0.3	1.5±0.2	1.9±0.1
Holostemma ada-kodien	1±0.2	1.3±0.2	1.8±0.3	2.1±0.3
Decalepi shamiltonii	1±0.1	1.2±0.1	1.6±0.1	2.3±0.2
Gloriosa superba	1.1±0.1	1.6±0.4	1.9±0.2	2.3±0.3
Gymnema sylvestre	1±0.2	1.3±0.3	1.7±0.1	2.2±0.4
Santalum album	1±0.5	1.6±0.5	1.9±0.3	2.3±0.1

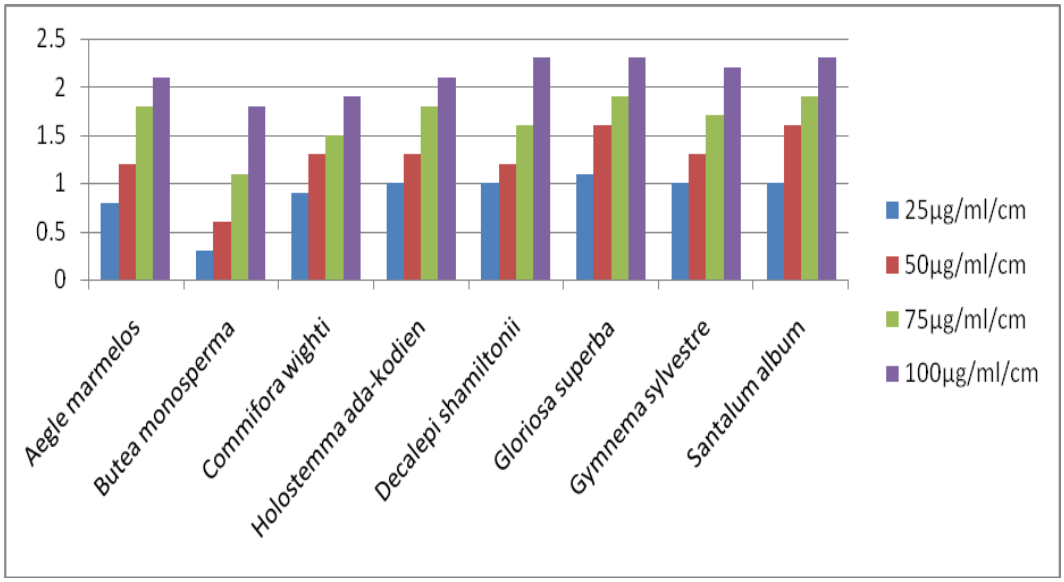

Fig 2: Antibacterial activity of selected medicinal Plants against *K. pneumonia*

Table 3: Zone of inhibition of antibacterial activity of selected medicinal plants against *P. aeruginosa*

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	0.4±0.2	0.7±0.2	1.1±0.2	1.5±0.2
Butea monosperma	0.6±0.1	0.9±0.2	1.3±0.2	1.9±0.5
Commifora wighti	1±0.1	1.3±0.5	1.6±0.3	1.9±0.6
Holostemma ada-kodien	1.2±0.3	1.6±0.6	1.9±0.5	2.2±0.7
Decalepi shamiltonii	0.9±0.5	1.3±0.2	1.5±0.2	2±0.2
Gloriosa superba	0.8±0.1	1.3±0.1	1.5±0.3	2±0.5
Gymnema sylvestre	1±0.3	1.3±0.2	1.7±0.2	2.2±0.2
Santalum album	0.9±0.2	1.4±0.3	1.7±0.4	2.1±0.4

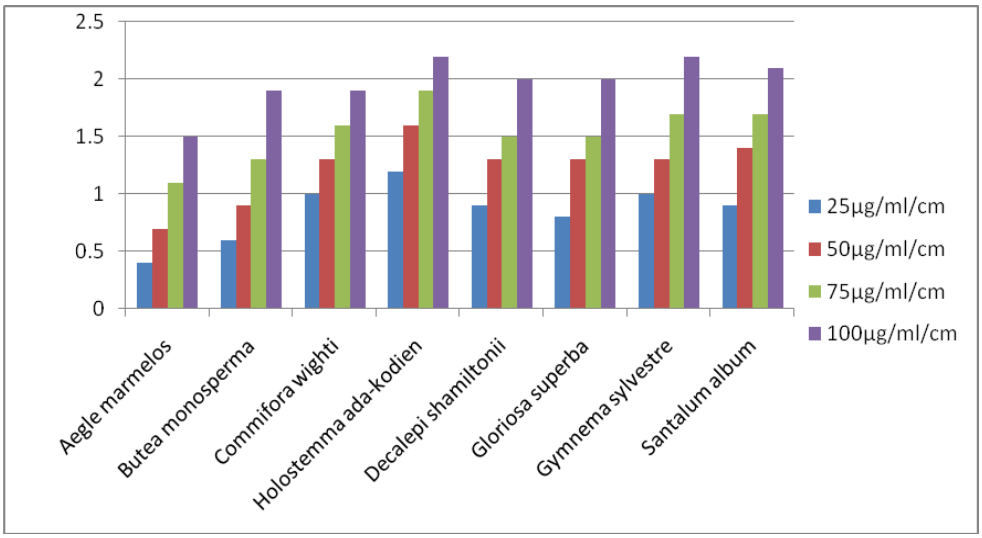

Fig 3: Antibacterial activity of selected medicinal Plants against *P.aeruginosa*

Table 4: Zone of inhibition of antibacterial activity of selected medicinal Plants against *P. vulgaris*

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	0.9±0.4	1.2±0.2	1.4±0.2	1.9±0.4
Butea monosperma	0.5±0.2	0.7±0.3	0.9±0.2	1.2±0.4
Commifora wighti	0.9±0.3	1.2±0.3	1.5±0.3	1.8±0.2
Holostemma ada-kodien	0.3±0.1	0.6±0.1	0.9±0.2	1.2±0.3
Decalepi shamiltonii	1±0.4	1.4±0.2	1.7±0.7	2.1±0.3
Gloriosa superb	1±0.5	1.5±0.2	1.8±0.5	2.2±0.2
Gymnema sylvestre	1.2±0.5	1.5±0.4	1.8±0.6	2.3±0.1
Santalum album	1±0.3	1.3±0.1	1.6±0.2	2.1±0.4

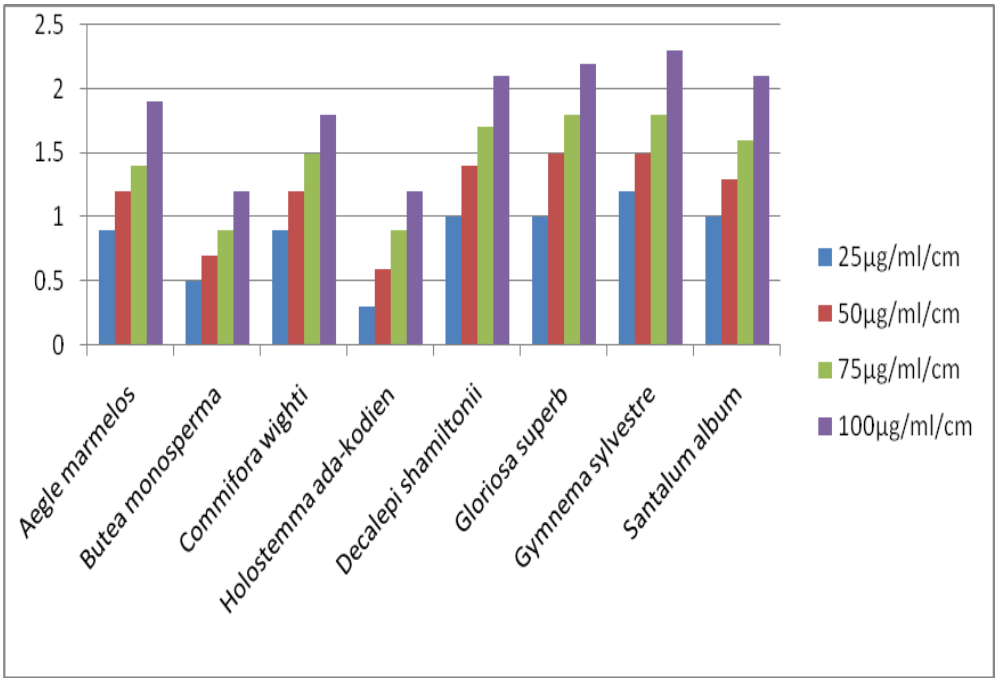

Fig 4: Antibacterial activity of selected medicinal Plants against *P. vulgaris*

Table 5: Zone of inhibition of antibacterial activity for selected medicinal plants against *B. subtilis*

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	1±0.2	1.2±0.4	1.5±0.4	2±0.1
Butea monosperma	0.8±0.5	1.1±0.2	1.5±0.1	2±0.4
Commifora wighti	1±0.1	1.2±0.1	1.5±0.5	1.8±0.6
Holostemma ada-kodien	0.5±0.2	0.9±0.1	1.2±0.2	1.5±0.4
Decalepi shamiltonii	1.1±0.3	1.6±0.5	1.9±0.4	2.2±0.7
Gloriosa superb	1.1±0.4	1.6±0.4	1.9±0.5	2.3±0.7
Gymnema sylvestre	1.1±0.2	1.5±0.4	1.9±0.5	2.2±0.4
Santalum album	1.2±0.4	1.5±0.3	1.9±0.2	2.2±0.4

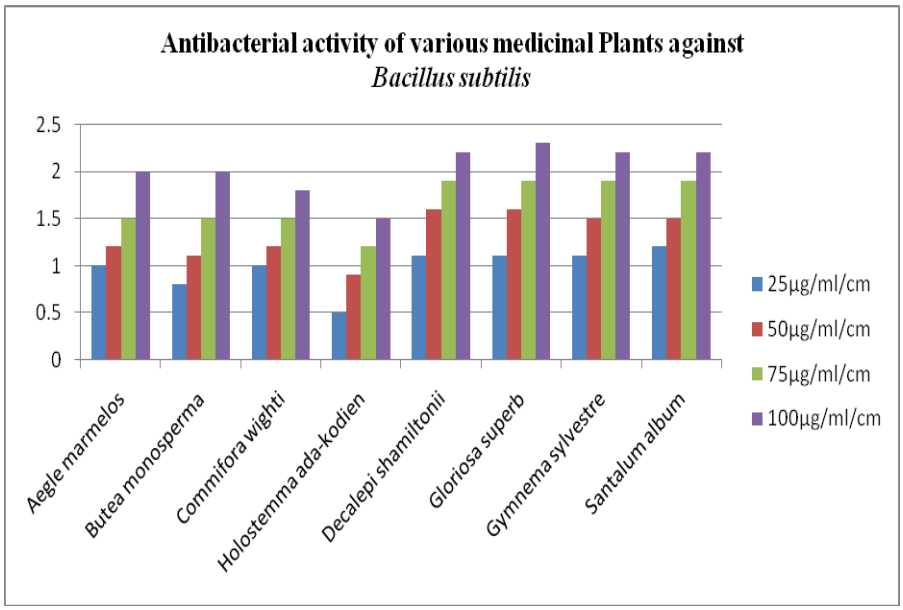

Fig 5: Antibacterial activity of selected medicinal Plants against *B. subtilis*

Table 6: Zone of inhibition of antibacterial activity of selected medicinal plants against *E. faecalis*

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	0.2±0.4	0.5±0.2	0.8±0.2	1.1±0.4
Butea monosperma	0.3±0.1	0.6±0.2	0.9±0.1	1.1±0.7
Commifora wighti	0.3±0.2	0.5±0.3	0.8±0.2	1±0.4
Holostemma ada-kodien	0.1±0.1	0.3±0.1	0.4±0.2	0.6±0.4
Decalepi shamiltonii	0.2±0.1	0.3±0.2	0.5±0.3	0.8±0.2
Gloriosa superb	0.5±0.2	0.7±0.1	0.9±0.2	1±0.1
Gymnema sylvestre	0.4±0.1	0.6±0.2	0.7±0.2	0.9±0.1
Santalum album	0.4±0.1	0.5±0.2	0.8±0.4	0.9±0.4

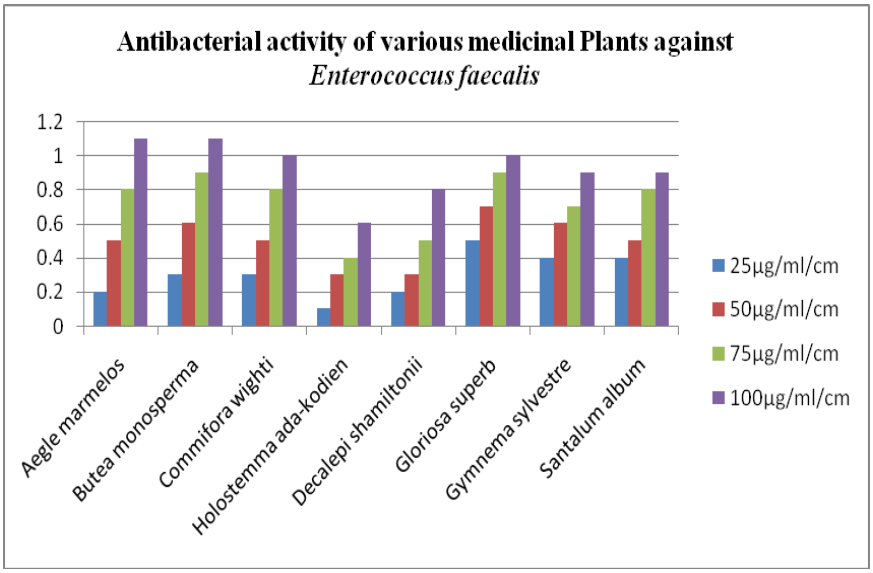

Fig 6: Antibacterial activity of selected medicinal Plants against *E. faecalis*

Table 7: Zone of inhibition of anti-fungal activity of selected medicinal plants against *C. albicans*.

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	0	0.2±0.1	0.5±0.1	0.8±0.4
Butea monosperma	0	0	0.2±0.1	0.4±0.2
Commifora wighti	0	0.1±0.1	0.3±0.2	0.2±0.1
Holostemma ada-kodien	0	0	0.1±1.1	0.3±0.2
Decalepi shamiltonii	0.1±0.1	0.3±0.1	0.4±0.2	0.6±0.1
Gloriosa superb	0.2±0.1	0.4±0.1	0.5±0.1	0.7±0.3
Gymnema sylvestre	0.3±0.1	0.4±0.1	0.5±0.3	0.8±0.3
Santalum album	0	0.1±0.1	0.2±0.1	0.3±0.2

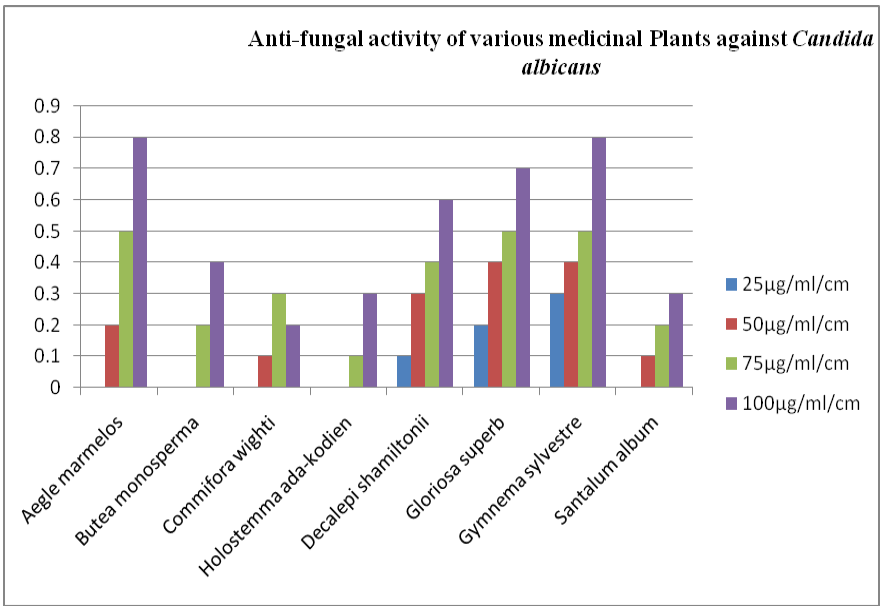

Fig 7: Anti-fungal activity of selected medicinal Plants against *C. albicans*

Table 8: Zone of inhibition of antifungal activity of selected medicinal plants against *A. niger*.

Plant name	25µg/ml/cm	50µg/ml/cm	75µg/ml/cm	100µg/ml/cm
Aegle marmelos	0	0.1±0.1	0.3±0.1	0.4±0.2
Butea monosperma	0	0	0.2±0.1	0.3±0.2
Commifora wighti	0	0.1±0.1	0.2±0.1	0.4±0.1
Holostemma ada-kodien	0.1±0.1	0.1±0.1	0.2±0.2	0.3±0.1
Decalepi shamiltonii	0.1±0.1	0.2±0.1	0.3±0.2	0.5±0.3
Gloriosa superb	0.2±0.1	0.4±0.2	0.5±0.3	0.7±0.4
Gymnema sylvestre	0.3±0.2	0.4±0.3	0.5±0.2	0.8±0.3
Santalum album	0	0.1±0.1	0.2±0.1	0.3±0.1

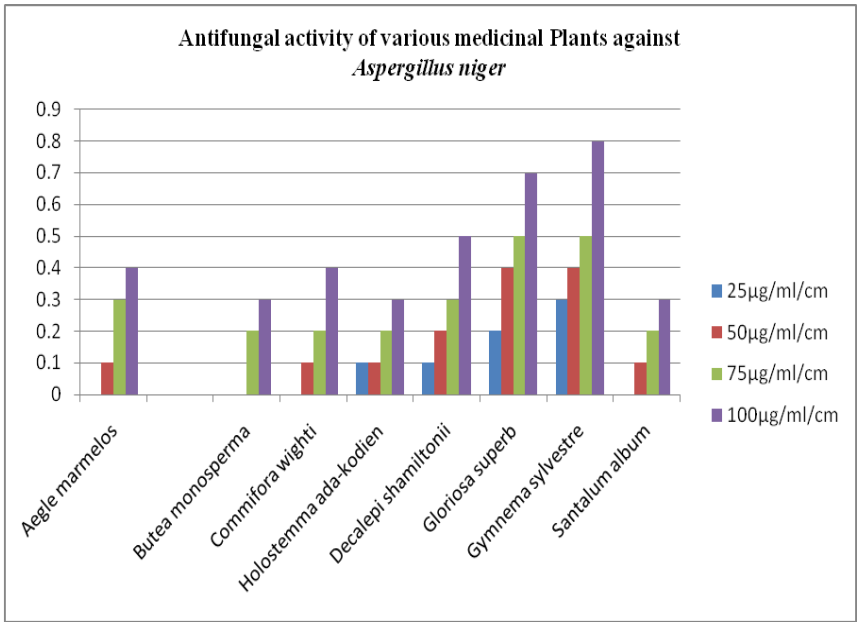

Fig 8: Antifungal activity of selected medicinal Plants against *A. niger*

The antimicrobial activity of *Gloriosa superb* against *E. coli* has given 1.3 cm zone of inhibition which is highest in the present study at concentrations of 25µg/ml/cm (low) and at a higher concentration of 100µg/ml/cm among all the plants studied.

Aegle marmelos showed higher zone of 3.5 cm. The Antimicrobial activity of *Gloriosa superba* leaf extracts against *Klebsiella pneumonia* has given a higher1.1 cm zone of inhibition at a low concentration of 25µg/ml/cm. Among all the plants and at a higher concentration of 100µg/ml/cm *Decalepis hamiltonii, Gloriosa superb* and *Santalum album* showed higher zone of 2.3cm. The antimicrobial activity of *Holostemma ada-kodien* leaf extracts against *Pseudomonas aeruginosa* has a higher 1.2 cm zone of inhibition at a low concentration of 25 µg/ml/cm among all the plants and at a concentration of 100 µg/ml/cm *Holostemma ada-kodien* and *Gymnema sylvestre* showed higher zone of 2.2cm. The activity of *Gymnema sylvestre* leaf extracts against *Proteus vulgaris has* given a higher 1.2 cm zone of inhibition at a low concentration of 25 µg/ml/cm among all the plants and at 100 µg/ml/cm concentration and also *Gymnema sylvestre* showed higher zone of 2.3 cm. *Gloriosa superba* leaf extracts against *Staphylococcus aureus* giving a higher 1.1 cm zone of inhibition at a low concentration of 25 µg/ml/cm among all the plants studied and at a higher concentration of 100 µg/ml/cm *Decalepis hamiltonii, Gloriosa superb* and *Santalum album* showed higher zone of 2.3cm. *Gloriosa superb* leaf extracts against *Enterococcus faecalis* has given a higher 0.5 cm zone of inhibition at a low concentration of 25µg/ml/cm among all the plants and at a higher concentration of 100µg/ml/cm *Aegle marmelos* and *Butea monosperma* showed higher zone of 1.1 cm.

Several studies conformed the *Candida albicans* and *Aspergillus niger* are resistant fungi, the present findings confirmed the anti fungal nature of these plant extracts and the methanol leaf extract of *Aegle marmelos, Gloriosa superba and Gymnema sylvestre* are effective. The present findings are similar to other studies *on Candida albicans* and *Aspergillus niger* (Uniyal *et al*., 2006, Bhadauria and Kumar, 2011) [12, 13].

The findings of the present study validates the leaf extracts of *Aegle marmelos*, *Butea monosperma*, *Commifora wighti*, *Holostemma ada-kodien*, *Decalepis hamiltonii*, *Gloriosa superba*, *Gymnema sylvestre* and *Santalum album* as medicinal in ancient medicinal system. The present study confirms the extracts of selected medicinal plants can be employed as antimicrobial agents in formulation of the novel drugs. Further it necessitates the pharmacological evaluation.

Conclusion

The bio active nature of the present selected medicinal plants may be due to the presence of various phytochemical components. The purified components may possess more activity rather than the crude extract.

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